Program/Abstract # 375
Two BMP ligands induce association of two nonredundant BMP Type I receptors to pattern the zebrafish dorsoventral axis
Shawn C. Little, Mary C. Mullins
Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA, USA

In both vertebrates and invertebrates, Bone Morphogenetic Protein (BMP) signaling patterns the dorsoventral (DV) axis. In the zebrafish embryo, DV patterning requires two nonredundant BMP ligands, Bmp2b and Bmp7, as well as the BMP Type I receptor Alk8 (the zebrafish ortholog of mammalian Alk2). We show that four additional genes encoding a distinct class of BMP Type I receptors, Alk3a/b and Alk6a/b, possess overlapping function and together are necessary for all BMP signaling during DV patterning. In contrast, Alk8 cannot substitute for the absence of Alk3/6; likewise, Alk3/6 cannot rescue *alk8* mutants. Thus, two classes of Type I receptor are nonredundantly required for DV patterning, as are two BMP ligands. A parsimonious model that accounts for these observations is that DV patterning requires BMP heterodimers signaling via receptor complexes containing one Alk8 and one Alk3/6 polypeptide, similar to a model of *Drosophila* DV patterning. To identify biochemical interactions of ligands and receptors in the zebrafish embryo, we injected functional epitope-tagged constructs at rescuing levels and analyzed interactions by immunoprecipitation. We find that both homo- and heterodimers form in the embryo. Importantly, Alk3a and Alk8 interact in wild-type embryos, but only in the presence of both Bmp2b and Bmp7. Based on these findings, along with previous biochemical and signaling analyses, we propose that heterodimers are necessary for efficient assembly of functional signaling complexes during DV patterning, due to BMP antagonists limiting ligand availability.

doi:10.1016/j.ydbio.2008.05.399

Program/Abstract # 376
BMP signaling progressively patterns the dorsoventral axis from anterior to posterior
Jennifer A. Tucker [a,b], Keith A. Mintzer [a], Mary C. Mullins [a]
[a] *University of Pennsylvania, Philadelphia, PA, USA*
[b] *NYU School of Medicine, New York, NY, USA*

The patterning of cell fates along multiple axes must be precisely coordinated in the early embryo. In vertebrates, patterning of the anteroposterior (AP) axis proceeds temporally from anterior to posterior. How this temporal progression is coordinated with cell fate specification along the dorsoventral (DV) axis has not been investigated. Current models of DV patterning by the Bone Morphogenetic Protein (BMP) gradient are static in the temporal dimension, with gradient function depicted at a single time point. However, proper interpretation of a gradient necessarily involves the aspect of time, as cells must not respond too early before a gradient is established, or too late after a gradient is perturbed by morphogenesis. Here, we examine the temporal activity of BMP signaling in patterning ventrolateral cell fates along the rostral AP axis. Using transgenes to rapidly turn BMP signaling 'off' or 'on', we show that BMP signaling prior to gastrulation provides little or no patterning information, whereas during gastrulation it patterns DV tissues in the ectoderm and mesoderm progressively in a cranial to trunk fashion. Rostral cranial DV cell fates are patterned by BMP signaling at the onset of gastrulation, while progressively more caudal cranial DV cell fates are patterned at progressively later discrete temporal intervals, rather than requiring longer exposure to BMP signaling. We propose a model whereby a temporal cue regulates the competence of cells to respond to BMP signaling, allowing the simultaneous acquisition of a cell's DV and AP identity.

doi:10.1016/j.ydbio.2008.05.400

Program/Abstract # 377
Tailbud-derived Bmp4 drives proliferation and inhibits maturation of zebrafish chordamesoderm
Robert Esterberg, Jean-Marie Delalande, Andreas Fritz
Biology Department, Emory University, Atlanta, GA, USA

In zebrafish, BMP signaling acts over multiple phases during gastrulation to establish mesodermal cell identity. Due to the early requirements of BMP activity in the patterning of the dorsoventral (D-V) axis, it has been difficult to assign later roles in cell fate specification to specific BMP ligands. Although the role of BMP ligands in promoting ventral mesodermal fate has been well established, the role of BMP activity in the development of axial structures is less clear. In this study, we have taken advantage of two *follistatin-like* genes (*fstl1* and *fstl2*), as well as a transgenic zebrafish line carrying an inducible truncated form of BMP-type I receptor to study the role of Bmp4 outside of the context of D-V specification. Characterization of *fstl1/2* suggests that they exert a redundant role as BMP antagonists beginning at late gastrulation. Although the loss of one or both Fstl proteins does not affect the gross morphology of the embryo, the diameter of the notochord is increased in embryos lacking Fstl1/2. BMP activity plays a direct role in the development of chordamesoderm, a subset of axial mesoderm that gives rise to the notochord, but not prechordal mesoderm, which gives rise to the prechordal plate. Sustained Bmp4 activity from the tailbud anlage is required during a critical window starting at late gastrulation and lasting through early somitogenesis to promote chordamesoderm proliferation. In the absence of Bmp4, the notochord precursor pool is depleted, and the notochord differentiates prematurely. Our results illustrate a role for Bmp4 in the proliferation and timely differentiation of axial structures.

doi:10.1016/j.ydbio.2008.05.401

Program/Abstract # 378
Fgfs in zebrafish left–right asymmetry
Michael R. Rebagliati [a,b], Nicholas Nedza [a], Timothy Eggleston [a], Gabriela Molina [c], Michael Tsang [c]
[a] *Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA, USA*
[b] *Stowers Institute for Medical Research, Kansas City, MO, USA*
[c] *Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*

The zebrafish charon gene encodes a Nodal antagonist that is needed to restrict nodal (southpaw) gene expression to left-side mesoderm. Loss of charon expression causes global left–right (LR) asymmetry defects. Charon is expressed in close association with Kupffer's vesicle (KV), a ciliated tissue required for normal LR patterning. We find that an fgf8 translation-blocking morpholino (MO) partially disrupts charon expression without disrupting formation of Kupffer's Vesicle. This contrasts with the published phenotype (C. Alberstson and P. Yelick) of the acerebellar allele, a zebrafish fgf8 mutation that disrupts fgf8 pre-mRNA splicing, KV formation and left–right asymmetry. General inhibition of LR signaling with SU5402